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NOTE

AUTOMATED SIMULTANEOUS DETERMINATION OF SERUM TOTAL PROTEIN AND GLOBULIN

P. Z. Sobocinski
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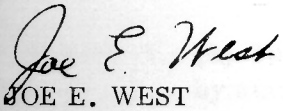

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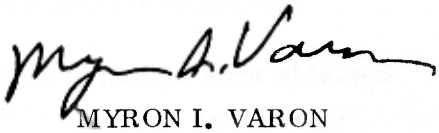

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ABSTRACT

An automated procedure for the simultaneous determination of serum total protein and globulin is presented. The total protein is determined by the biuret reaction while the serum globulin is determined by the reaction of globulins with copper in the presence of sulfuric and acetic acids to form colored metalloprotein complexes. A major advantage of the proposed combined procedure is that the latter reaction is not influenced by the tryptophan content of serum and as such is applicable to the assay of globulin levels in patients with neoplastic disease. Details for flow manifold construction and reagent composition are presented together with data concerning the precision of the method.

I. INTRODUCTION

Present methods available for the automated determination of serum globulins employ the glyoxylic acid[2] or p-dimethylamino-benzaldehyde[4] reactions for tryptophan. A potential source of error in the determination of serum globulin levels by colorimetric procedures in patients with neoplastic disease is the variations encountered in the free- and bound-tryptophan content of these sera.[3]

This study was undertaken to develop an automated simultaneous procedure for the determination of serum total protein and globulin in which the globulin assay was independent of tryptophan content of serum.

In a preliminary study, it was found that the manual procedure[1] employing the formation of metallo-organic complexes was not significantly influenced by tryptophan. We therefore selected this system for automation. Since there was no apparent reason to question the validity of the well-established biuret reaction for measuring total protein, this procedure was adapted essentially as described by Savory et al.[2] for automation.

This report presents details for flow manifold construction and data concerning the accuracy and precision of the proposed method.

II. MATERIALS

All reagents were obtained commercially (Hycel, Inc., Houston, Texas) as follows: (a) stable biuret reagent, No. 201A consisting of 0.15 percent $CuSO_4$ and 0.6 percent $NaKC_4H_4O_6$ in 3 percent NaOH as described by manufacturer; and (b) globulin reagent, No. 197 consisting of 0.08 percent $CuSO_4$ and 6.55 percent H_2SO_4 in 81.6 percent CH_3COOH as described by manufacturer.

III. PROCEDURE

The flow diagram for the simultaneous procedure using the AutoAnalyzer (Technicon Corporation, Tarrytown, New York) is shown in Figure 1. Serum sa are aspirated at a rate of 60 per hour with a water wash sample between serum sa ples to obtain optimum resolution between sample peaks. The serum sample is su quently separated by a stream splitter into two separate flow manifolds, total prote and globulin. The amount of colored products formed in both reactions is measure

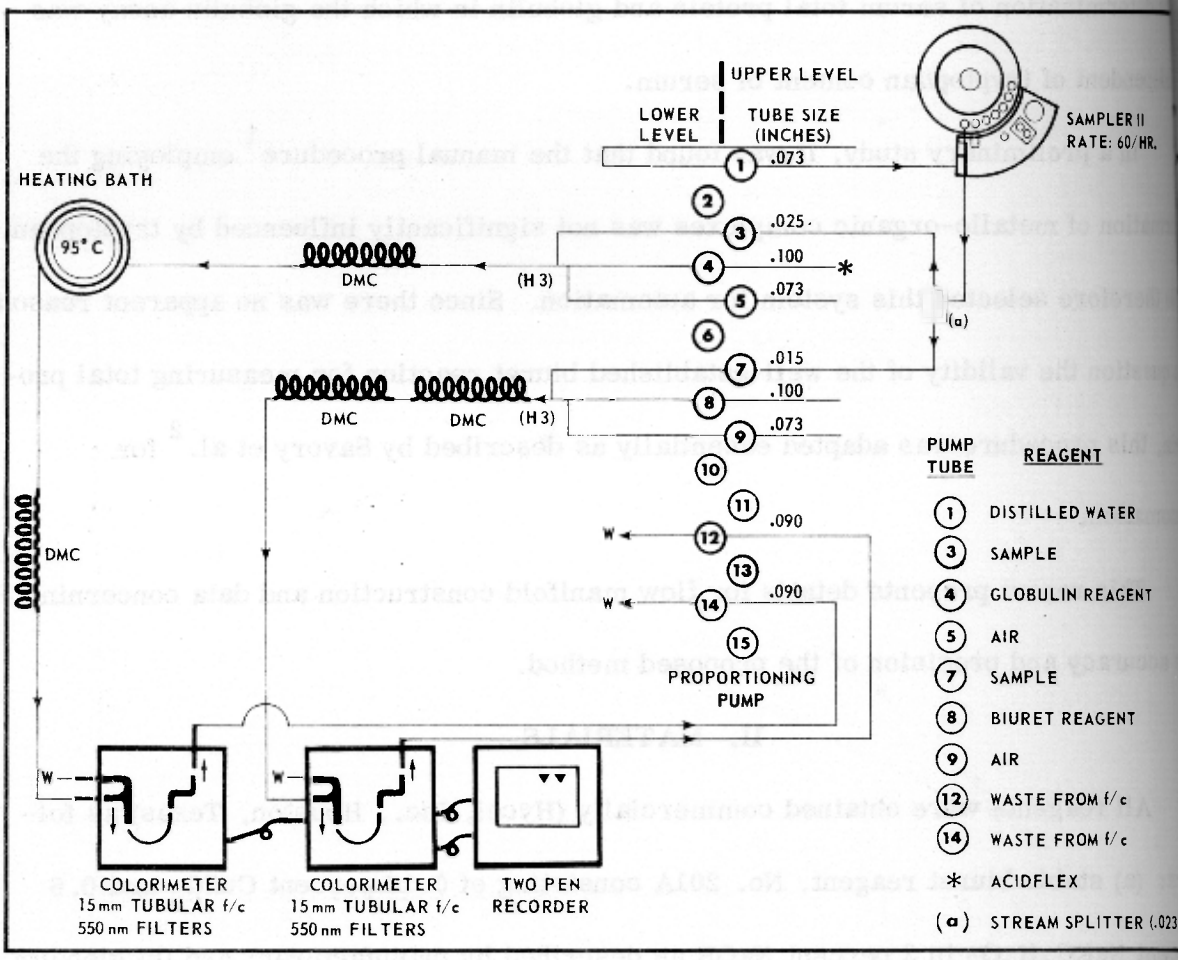

Figure 1. Diagram of the flow manifold for the automated simultaneous determination of serum total protein and globulin. Glass fittings and tube sizes are Technicon designations.

at 550 nm. The concentration of each serum constituent is determined from a calibration curve prepared from known human control serum.

Because of the corrosive reagent used in the globulin determination all flexible tubing in the globulin manifold must be Acidflex (Technicon). In addition, this reagent must be protected from atmospheric moisture[1] by inserting a drying tube in the reagent receptacle.

IV. RESULTS AND DISCUSSION

A typical calibration curve for the simultaneous procedure is shown in Figure 2. Linearity is obtained within the concentration range of 0.0 to 10.0 g/dl total protein and 0.0 to 3.9 g/dl globulin. Serum samples containing total protein-globulin levels in excess of these limits should be diluted with 0.9 percent NaCl prior to final analysis.

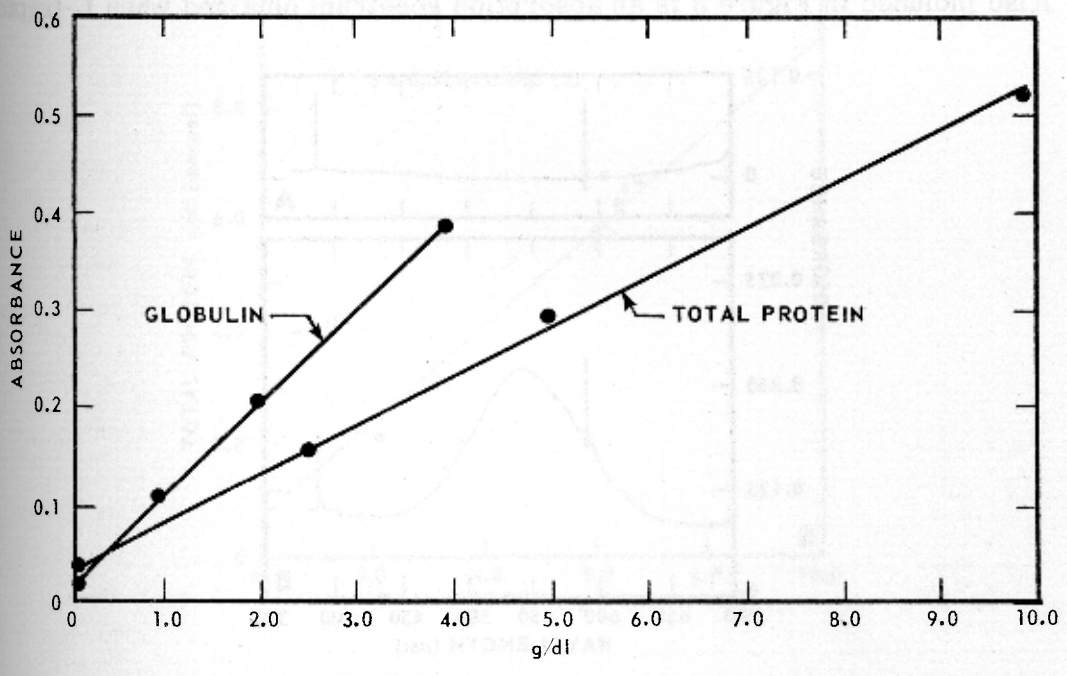

Figure 2. Calibration curves for the determination of serum total protein and globulin by the proposed automated simultaneous method

Physiologic saline is used instead of distilled water since color production is optim within physiologic saline concentrations.[1]

Results of 20 replicate analyses performed on a commercial control serum (Versatol-A Lot No. 0572097, Warner-Chilcott Laboratories, Morris Plains, New Jersey) indicate that the standard deviation for the total protein determination is ± 0.10 g/dl and for the globulin determination is ± 0.05 g/dl. The reported standard deviation values for the manual procedure are as follows: Total protein, ± 0.5 g/dl and globulin, ± 0.4 g/dl.[1] It is obvious that the proposed automated procedure considerably reduces the standard deviation for each method.

The absorption spectrum obtained for the products of the reaction with serum globulins is shown in Figure 3. Maximum absorption occurs at approximately 545-55 nm. Also included in Figure 3 is an absorption spectrum obtained when L-tryptopha

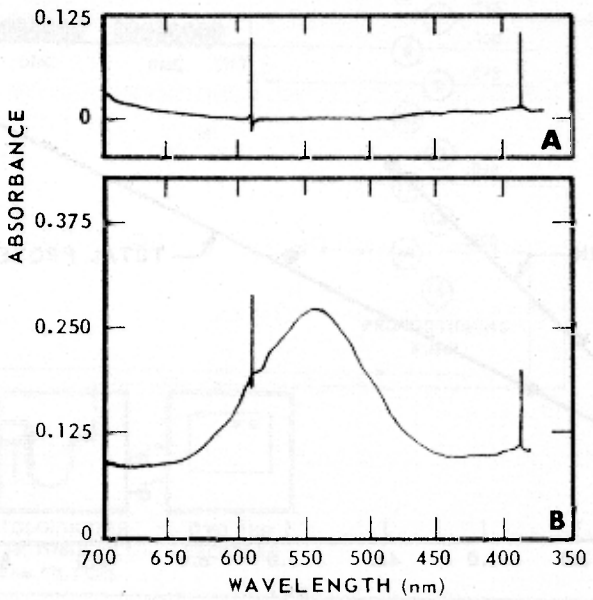

Figure 3. Absorption spectra of products obtained for (A) L-tryptophan (1.0 g/dl) an (B) human serum in the proposed automated method. Vertical lines in the recording are due to filter changes in the spectrophotometer (Acta II, Beckman Instruments, Inc., Fullerton, California).

(1.0 g/dl) is reacted with the globulin reagents. This concentration of tryptophan is far in excess of that observed in cancer patients.[3] These results clearly demonstrate that tryptophan does not react with the globulin reagents.

Results of a correlation study between globulin and total protein values in human sera obtained by manual and automated procedures are shown in Figure 4. The

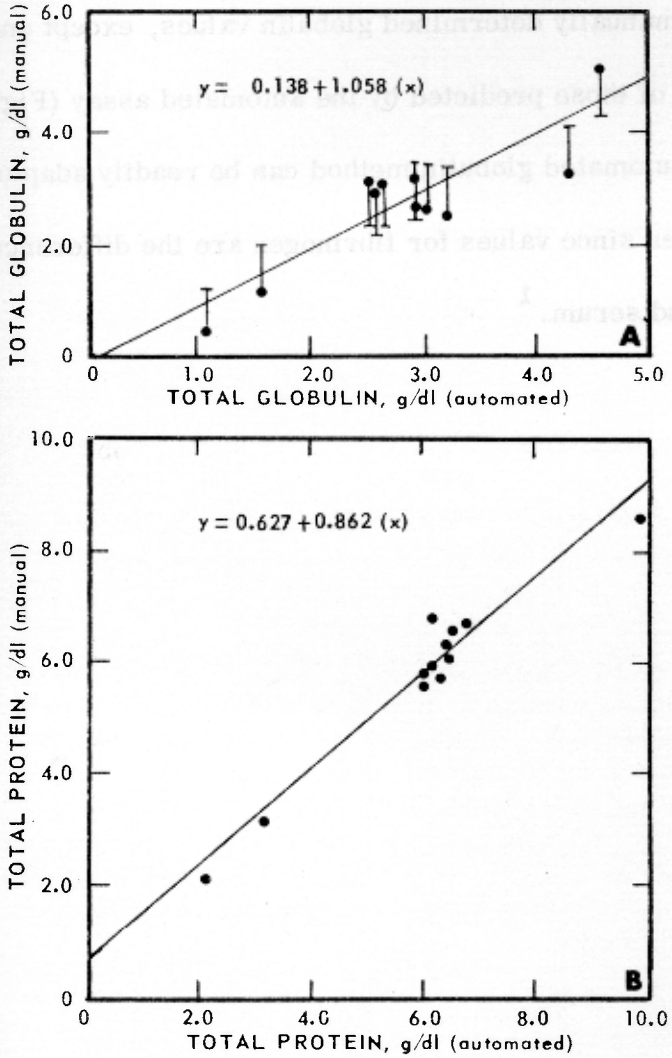

Figure 4. (A) Total globulin and (B) protein levels in human sera obtained by automated and manual procedures. Vertical brackets indicate ± 2 S. D. for manual procedure. Equations for each line are shown. Correlation coefficients (r) are stated in the text.

correlation coefficient (r) was found to be 0.874 for globulin and 0.975 for total protein determinations. These results indicate that there is good agreement between total protein values obtained by manual and automated procedures. The correlation obtained for globulin values is somewhat lower than that obtained for total protein values, due primarily to the greater standard deviation for manual globulin determinations compared to automated. All manually determined globulin values, except one, are within two standard deviations of those predicted by the automated assay (Figure 4, curve A).

Finally, the automated globulin method can be readily adapted for the automated analysis of fibrinogen since values for fibrinogen are the differences between globulin assays of plasma and serum.[1]

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